Research Article

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Histopathological Study of the Liver in Balb/C Mice with Lupus Treated by Bone Marrow-Derived Mesenchymal Stem Cells (Bm-Mscs)

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Abstract

Lupus is an autoimmune disease in animals and humans, characterized by THE production autoimmune antibodies against its cell proteins. The present study investigates the liver tissue changes in BALB/c mice with lupus and investigates the effects of bone marrow-derived mesenchymal stem cells (BM-MCSs) for treatment of tissue pathological changes. We divided forty BALB/c mice into four equal groups for the study. G1 included mice with lupus. G2 included mice with lupus, which were then treated by BM-MCSs. G3 are administered BM-MCSs alone. G4 administered PBS. After ending the treatment periods, all the animals were sacrificed. The liver was placed in buffered formalin at 10% and sent to the histopathology laboratory to determine the histopathological changes in all study groups. The histopathological changes of G1 are liver severe necrosis with a atrophy, hydropic liver cells, very wide sinusoids, severe hemorrhage, and an engorged central vein. G2 showed normal liver cells with a slight necrosis. G3 and G4 showed the liver tissue's normal architecture. Lupus causes histopathological changes in the mice's liver; MSC can ameliorate and treat the liver histopathological changes in BLAB/c mice with lupus.

Keywords: Examination, liver, lupus, MSCs

Introduction:

Lupus is an autoimmune disease where the body's immune system targets healthy tissue as a result of attacking its own tissues. Lupus causes inflammation in many different tissues and organs, such as heart, skin, joints, lungs, and liver (1). Lupus is

one of the autoimmune diseases that occur in animals and humans and causes various degrees of inflammation (acute, subacute, and chronic) in most body tissues. The liver is affected by lupus and shows acute inflammation (2). Lupus causes autoimmune inflammation in most organs, including the liver. Furthermore, systemic lupus erythematosus causes an elevation of liver enzymes. Lupus can also cause hepatic vasculitis inflammation of the blood vessels that are related to the liver, and blood clots (3).

Some of the patients with lupus showed inflammation in the liver. In both children and adults, lupus hepatitis is linked to lupus The liver biopsy is the main way to detect lupus hepatitis.

Typically, the inflammation is mild. Sometimes, lupus hepatitis leads to fatigue, appetite loss, pain, abdomen fluid, and cirrhosis (4,5). Lupus causes several histopathological changes in the liver, such as hepatitis, hepatitis, lymphoplasmacytic infiltrate, plasma cell infiltration, periportal fibrosis, nonspecific inflammatory infiltrate in the portal tracts and lobules, focal necrosis, lobular inflammation, vasculitis, thrombosis, infarction, hemorrhage, cholangiopathy, bile duct damage, and periductal fibrosis (6).

Liver involvement in lupus showed abnormal liver enzymes, liver function abnormalities, a fatty liver, and chronic hepatitis (7).

SLE causes histopathological damage to the liver, which includes hepatitis, cirrhosis development, steatosis, liver lesions, blood vessel lesions, and hepatotoxicity (8). In the first stages, lupus causes hepatic necrosis in women. SLE also causes abortions in pregnant women because of increased serum anti-nuclear antibodies and anticardiolipin antibodies (9). A Abnormalities in liver functions and histological changes are associated with SLE.Jaundice, liver enlargement, cirrhosis, and vascular changes occur at different stages of lupus development (10). Cirrhosis is associated with lupus, but it is rare. When cirrhosis is occurring, a bad prognosis is indicated, which means an advanced stage of lupus (11). The study investigates the histopathological changes of the liver in mice with lupus. BM-MSCs have the potential to treat various diseases.

Materials and Methods

The experimental animals

Forty mice at six weeks old, with (25) grams on average, were provided by (Jackson Laboratory, USA) and housed together.

The study design

We have four groups of BALB/c mice; G1 is given Activated Lymphocyte-Derived DNA (50 µg/mouse) at (0, 14, 28) days (three doses). G2 is given Activated Lymphocyte-Derived DNA (50 µg/mouse) at (0, 14, 28) days (three doses); after the clinical signs manifest, the antinuclear antibody ANA, and anti-dsDNA are determined. The positive ANAand anti-dsDNA SLE in G2 treated by MSCs (Cell Biologics company, USA) (100.000) cells/for 10g/IV. G3 was given MSCs (100.000) cells/for 10g/IV. G4. The

G4 (control group) given BPs. The induction of SLE is done according to (12).

ANA and anti-dsDNA examination:

Eliza kit ANA and Eliza kit anti-double strand DNA (MyBioSource, USA) are used for the detection of antibody levels. BM-MSCs are provided by (Cell Biologics Company, USA). It is prepared according to the company's directions. G2 received BM-MSCs at a dose of (100.000) cells (IV) per 10 grams.

The histopathological examination:

The tissue processing involves several steps. Here are the general steps involved in this process:

1-Fixation: The tissue sample is first collected and immediately placed in a fixative solution, which preserves the tissue structure and prevents degradation. Common fixatives include formalin, ethanol, and methanol.

2-Dehydration: The tissue sample is then dehydrated by being immersed in a series of alcohol solutions with increasing concentrations (e.g., 70%, 80%, 90%, and 100% ethanol) to remove the water from the tissues.

3-Clearing: the tissue is cleared by xylene to remove alcohol.

4-Infiltration: The tissue is put in the paraffin wax to support the tissue.

5-Embedding: The tissue is put into molds of the paraffin wax to solidify for sectioning.

6-Sectioning: The block is cut into (4-8) microns in thickness by the microtome, and then put on the glass slides.

7-Staining: This step is done by using two stains hematoxylin and eosin, to contrast the different structures of the tissue. The cover slide put over the stained slide to make it easy to see under microscope.

Results

G1 showed severe liver necrosis, degenerated liver cells, very wide sinusoids, severe hemorrhage, liver cell loss, striation, engorged central vein, and liver atrophy, as shown in Figures (1) and (2).

According to G2, the liver tissue is normal, with a small area of necrosis and little eosinophilic cytoplasm, as shown in Figures (3) and (4). G3 showed the normal architecture of the liver tissue, as shown in Figure (5). Finally, G4 showed that the liver hepatocytes have normal shape and architecture, as shown in Figures (6) and (7).

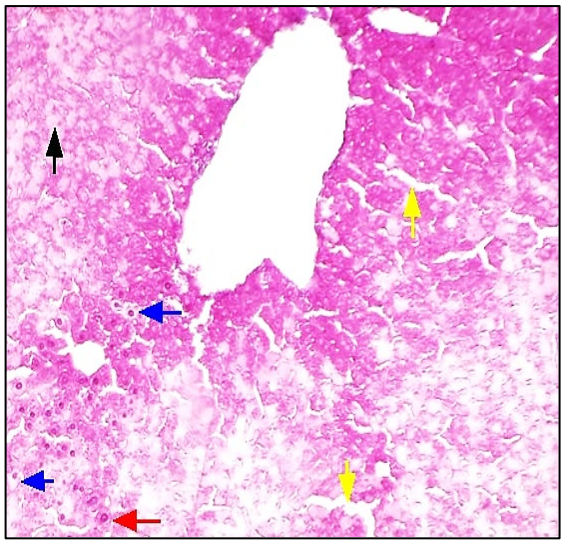

Figure (1): shows liver severe necrosis (black arrow), degenerated liver cells (blue arrows), and wide sinusoids (yellow arrows) with some viable cells (red arrow). H&E, 100X (G1).

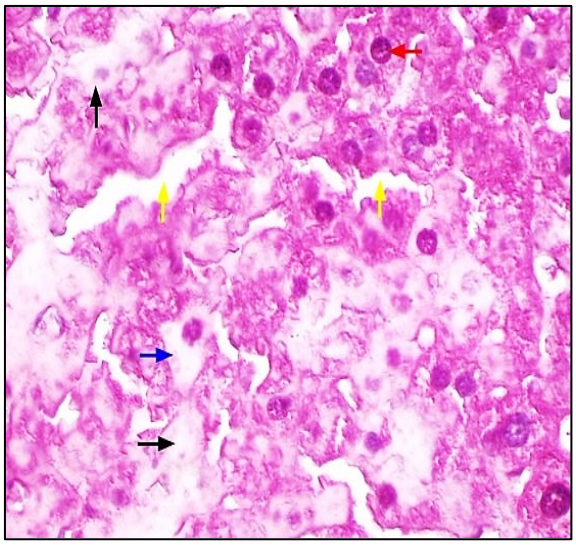

Figure (2): shows liver necrosis (black arrows), degenerated liver (blue arrow), wide sinusoids (yellow arrow), and viable cells (red arrow). H&E, 400X (G1)

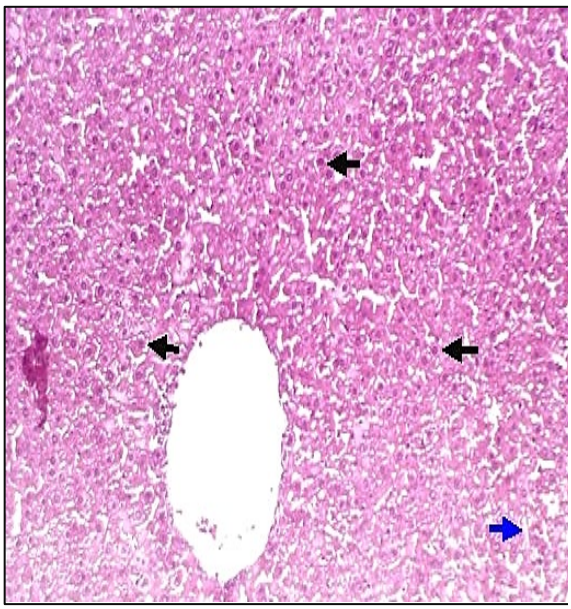

Figure (3): shows normal liver cells (black arrows), with some focal area of necrosis (blue arrow), and wide sinusoids. H&E, 100X (G2).

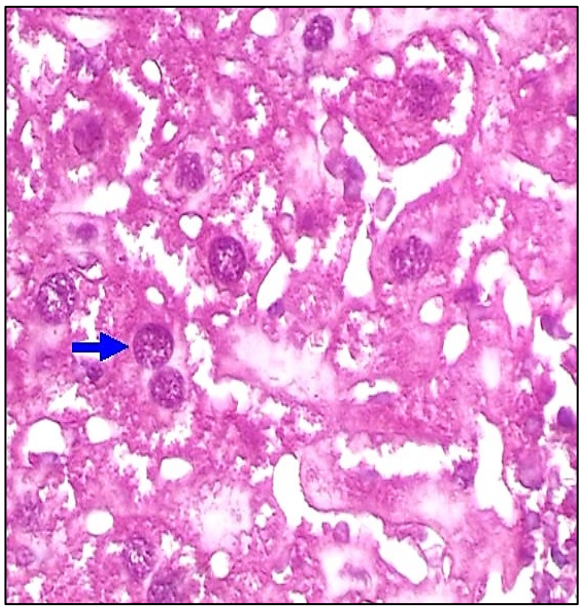

Figure (4): shows normal liver cells, with little eosinophilic cytoplasm, some necrotic liver cells and wide sinusoids. H&E, 400X (G2).

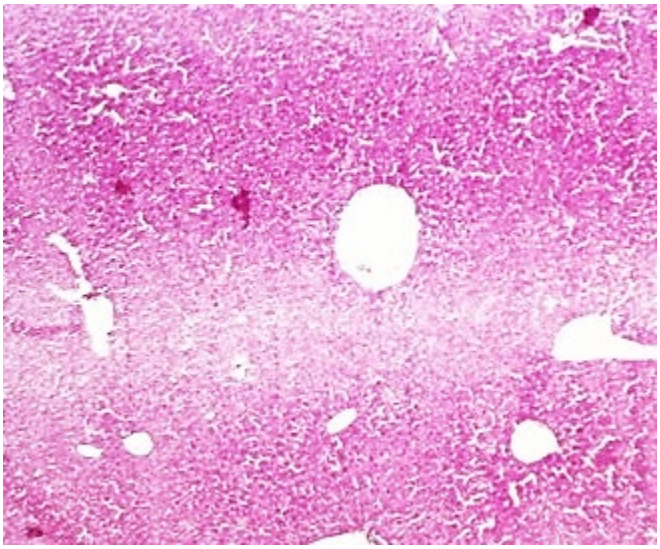

Figure (5): shows normal liver architecture in the liver section. H&E, 40X (G3).

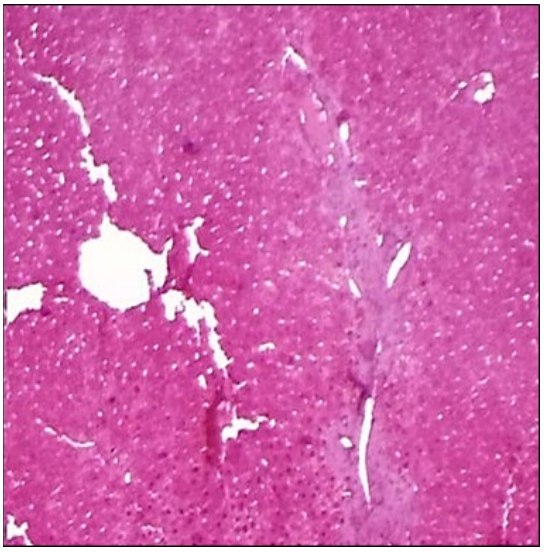

Figure (6): shows normal liver. H&E, 40X (G4)

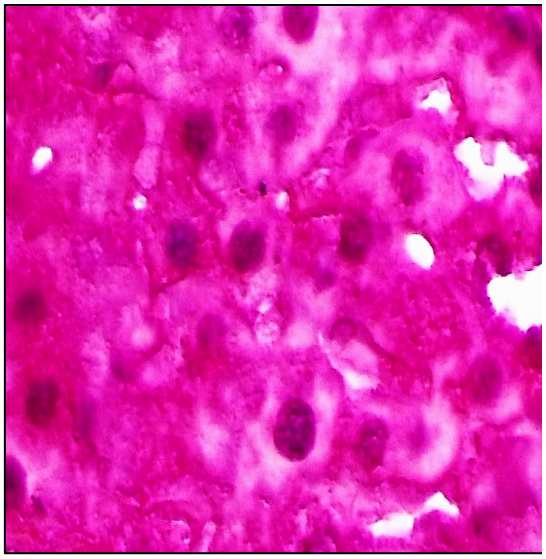

Figure (7): shows normal liver. H&E, 400X (G4).

Discussion

According to the findings, G1 showed severe necrosis of liver tissue, degenerating liver cells, wide sinusoids, severe hemorrhage, loss of liver tissue striation, liver atrophy, and engorgement of the vein. In a study conducted in Japan on patients diagnosed with lupus, several histopathological changes of the liver were discovered. These changes included liver congestion, fatty liver, blood vessel inflammation of the liver, liver cells hyperplasia, chronic hepatitis, and hemangioma. In addition, the formation of nodular regenerative hyperplasia is seen in the liver of patients who have lupus symptoms (20). In a study that included 47 individuals with lupus who had liver function disorders and tissue abnormalities, the patients with active lupus had the highest incidence of hepatitis. These patients have higher leucocyte and platelet counts than healthy individuals (21).

According to our results, the patients with treated lupus with MSCs showed liver necrosis with most common normal hepatocytes, whereas the sinusoids were slightly dilated. In large comparing of mesenchyme stem cells used in the therapy of hepatitis in lupus, the advantage is that MScs can differentiate into a variety of cell type and possess immunomodulation effects. They achieve this by rebuilding the lost liver tissues, as well as modulating the immune system to avoid autoimmune inflammation. Therefore, MSCs possess the ability to regenerate damaged tissue, thereby halting the progression of hepatitis and fostering liver restoration (22). MSCs also carry immunomodulatory properties and capacity to control immune functions through the process of proliferative arrest of immune stromal cells, including T cells and B cells. Also, they have the property of lowering the level of aqueous humor of the pro-inflammatory cytokines. Therefore, MSCs possess the ability to regenerate damaged tissue, thereby halting the progression of hepatitis and fostering liver restoration (22). MSCs also possess immunomodulatory properties and the ability to control immune functions through the proliferative arrest of immune stromal cells, including T cells and B cells. They also have the property of lowering the level of pro-inflammatory cytokines in the aqueous humor (23).

In addition to being multipotent stem cells, they release a number of proteins and chemicals for human growth and tissue repair. These molecules were identified to enhance the regeneration of liver infrastructure and the angiogenesis of intrinsic liver cells, in addition to the management of inflammation cycles in the injured liver (24). MScs contributed to the registration of mitosis as well as apoptosis, whereas they also contributed to the synthesis of VEGF and CXCL-12, participating in the reconstruction of the damaged tissue (25They discovered that MSCs have the procedural capability to differentiate down to almost all stages of cellularity. Particularly, we have defined the

fundamental principles regulating MSCs and provided examples of their involvement in tissue alterations and immune system control. Some of the diseases for which mesenchymal stem cells (MSCs) may be used for the treatment are multiple sclerosis, lupus, rheumatoid arthritis, and many others (26).

Lupus could be the other potential of regeneration, wherein MSC could be used for the repair of the damage in the liver. Nonetheless, research completed in the present day reveals the fact that MSCs can suppress the immune system. Leucosis, on the other hand, is referred to as a disease that compromises the immune system of a human being through infecting healthy tissues like the liver, among others. On one front, MSCs could potentially down-regulate the immune response thereby tackling inflammation, which leads to liver damage (27). As previously discussed, stem cells discharge a large number of anti-inflammatory molecules, whereas multiple sclerosis cells also discharge anti-inflammatory entities, thereby assisting in the attenuation of inflammation in the liver. These stem cells can also be capable of releasing cytokines that would be useful for the maturation of the others into liver cells each time some of them are injured (28). SLE was associated with an elevation in liver diseases, which comprises aspects such as histological features, liver function, and pathological signs. The data outlined above were believed to have been in concordance with the literature cited in our study, which focused on hepatic histopathological alterations that result from lupus. This might be because of the characteristic of MSCs of transforming into hepatocyte-like cells after going through maturation, then differentiating and assimilating into the liver parenchyma (29). Granulation tissue that consists of connective tissue and blood vessels may form in the liver parenchyma as a consequence of liver injury, and may impair liver function. A particular type of cells called a multipotent stem cell has properties of decreasing fibrosis, encouraging the formation of extracellular matrix, and enhancing tissue healing processes (30).

Conclusion

SLE is a systemic disease that changes the liver histopathology in BALB/C mice. The changes in the liver histopathology included hepatocyte degeneration and necrosis, dilated sinusoids and severe bleeding, and hepatocyte atrophy. The study used the BLAB/C mouse model of SLE to show that the mesenchymal stem cell works better and can fix changes in the liver's histopathology.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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الاحمراري والمعالجة بالخلايا الجدعية الوسيطة المشتقة من نخاع العظم (BM-MSCS)

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الخلاصة

الذئبة الاحمراري مرض مناعي ذاتي يصيب الحيوانات والبشر، ويتميز بإنتاج أجسام مضادة مناعية ذاتية ضد بروتينات خلايا الجسم. تبحث هذه الدراسة في التغيرات التي طرأت على أنسجة الكبد لدى فئران BALB/C المصابة بمرض الذئبة، كما تبحث في تأثيرات الخلايا الجذعية الوسيطة الوسيطة المشتقة من نخاع العظم (BM-MCSs) لعلاج التغيرات المرضية التي طرأت على الأنسجة. شملت الدراسة أربعين فأرًا من فئرانBALB/C ، وقسمت الحيوانات إلى أربع مجموعات بالتساوي. شملت المجموعة G1 الفئران المصابة بالذئبة G2 .شملت الفئران المصابة بمرض الذئبة، ثم عولجت بخلايا .أُعطيت المجموعة G3 فئران مصابة بالذئبة BM-MCSs فقط أما المجموعة G4 فقد اعطيت PBS . بعد انتهاء فترات العلاج، تمت التضحية بجميع الحيوانات. تم وضع الكبد في الفورمالين بتركيز 10% وأرسلت إلى مختبر التشريح المرضي لتحديد التغيرات المرضية النسيجية في جميع مجموعات الدراسة. كانت التغيرات المرضية النسيجية في المجموعة G1عبارة عن نخر شديد في الكبد مع ضمور وخلايا كبدية مائية وجيوب جيبية واسعة جداً ونزيف شديد ووريد مركزي محتقن. أظهر G2 خلايا كبد طبيعية مع نخر طفيف. أظهر G3 و G4 بنية طبيعية لأنسجة الكبد. يسبب مرض الذئبة تغيرات نسيجية في كبد الفئران؛ وبالامكان أن يخفف MSC من التغيرات النسيجية المرضية للكبد ويعالجها في فئران BLAB/c المصابة بالذئبة .

الكلمات المفتاحية: الفحص، الكبد، الذئبة، الخلايا الجذعية المساريقية.